

Exploring the Antagonistic Potential of Fungal Endophytes isolated from *Andrographis paniculata* (Burm. f.) wall. Ex Nees and Qualitative Profiling of Secondary Metabolites from the plant extract

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Abstract: *Andrographis paniculata* is a noteworthy Ayurvedic herb that has antioxidant and anti-inflammatory properties and conventionally been used to deal various liver-related diseases. Thus, this study concentrates on isolation and identification of endophytic fungi from different parts of this plant, assess their antimicrobial activity, and examine the presence of phytochemicals, in order to evaluate the plant's potential as a source of bioactive compounds for pharmaceutical or agricultural applications.

Research methods: For the isolation of endophytic fungi, the plant sample has been fragmented from various parts of the plant, such as- from leaf, stem and roots and each fragment then surface sterilized with the procedure given by Bayman [4]. Treated fragments were inoculated on Potato Dextrose Agar (PDA) and Czapek Dox Agar (CDA) plates and incubated at $25 \pm 2^\circ\text{C}$ for 7-10 days. Antagonistic activity of the fungal isolates against selected pathogens were measured using dual culture method given by Talapatra [39]. The plant extract was used for the initial phytochemical screening using standard protocols from earlier studies [12; 3; 36; 37].

Findings and values: The three dominant fungal endophytes from *A. paniculata* were isolated: *Aspergillus* sp. (LP1- from leaf fragment), *Colletotrichum* sp. (SP1-from stem fragment), *Penicillium* sp. (SR1- from root fragment). In dual culture assessment it has been found that *Aspergillus* and *Penicillium* spp. hinder the growth of *Escherichia coli*, whereas *Colletotrichum* sp. completely overgrew the bacterial pathogen *Escherichia coli*. The phytochemical screening confirms the existence of phenols, alkaloids and flavonoids in the stem sample whereas alkaloids and flavonoids are present in the leaf sample. Tannin and saponin were absent in both the parts. Thus, these findings support the plant's potential as a bioresource for antimicrobial and bioactive compounds.

Keywords: *Andrographis paniculata*; Fungal endophytes; Antimicrobial activity; Phytochemical screening; Dual culture method.

1. Introduction:

Andrographis paniculata (Burm.f.) Wall. ex Nees., also known as "Kalmegh" or "Green Chiretta" is a medicinal herb extensively used in traditional medicine, predominantly in Ayurvedic and Traditional Chinese as well as Indian Medicine. Acknowledged for its bitter taste, the plant is often referred to as the "King of Bitters." The active compounds in *Andrographis paniculata*—primarily andrographolides—have shown various health aids due to their anti-inflammatory, antioxidant, and antimicrobial properties. Its extracts exhibit a wide range of bioactivities, including antioxidant, immunostimulatory, antidiabetic, anti-infective, anti-angiogenic, sex hormone-modulating, antimicrobial, cytotoxic, antiparasitic, anti-inflammatory, hepatomodulatory, insecticidal, and toxicological effects. [23]. *Andrographis* is a genus within the family Acanthaceae, comprising approximately 40 species. Among them, *Andrographis paniculata* is the most widely recognized due to its extensive use in traditional medicine. It is an erect, annual, and branched herb that typically grows 0.5–1 m in height. Originally endemic to the Indian subcontinent—particularly Peninsular India and Sri Lanka—it has since become widely distributed across Southeast Asia, China, the Americas, the West Indies, and Christmas Island. The aerial parts of the plant possess notable medicinal properties and contain a diverse array of phytochemicals, including flavonoid diterpenoids, diterpene glycosides, flavonoids, and other glycosides. Well-designed clinical trials have demonstrated its safety and efficacy in alleviating symptoms associated with common upper respiratory tract infections [2]. Apart from having extensive medicinal properties, this plant harbours a wide range of endophytic

fungi which lives within plant tissues without causing any harmful effect. They build a beneficial symbiotic association, enhancing plant growth, stress tolerance, and defence against pests and pathogens. Found in diverse ecosystems, these fungi contribute to nutrient cycling, produce growth-promoting compounds, and play key roles in plant health. Growing research on endophytic fungi underscores their significant potential in agriculture, biocontrol, and biotechnology, largely due to their ecological diversity and beneficial interactions with host plants. However, our current understanding of endophyte diversity remains limited [32]. Many types of endophytic microorganism, lives inside plant tissues throughout their lives or during a certain period of their life cycles and causes no visible damage or morphological changes. They are potential sources of novel natural agents for manipulation in the pharmaceutical industry, agriculture, and in environmental applications. Endophytic fungi has the ability to produce the same metabolic components as the host plant as it can obtain certain matching genes of the host by feasting on the host DNA and also act as initiators to swiftly activate specific genes in the secondary metabolic pathways of medicinal plants [33]

2. Materials and Methods:

2.1 Sample collection:

For the present investigation, the plant samples were collected from Kamarkuchi village, Sonapur, Assam (India) in a sterile polyethene bags and were directly transported to the laboratory. They were stored in the refrigerator at 4°C for further use.

2.2 Isolation and characterisation of fungal endophytes:

The surface sterilization was performed using the slight modified protocol given by Bayman and his associates [4]. At first, the samples were rinsed properly under running tap water to remove the dirt and debris from the sample. Then stems, leaves and roots of the plant sample were chopped in small fragment (0.05 mm approx.) and dipped in 70% ethanol for 1 minute followed by treatment with 2% (v/v) sodium hypochlorite (NaOCl) for 3 minutes. Then re-immersing them in 70% ethanol for 30 second. After this, the samples were finally rinsed several times in sterilized distilled water to remove any traces of surface sterilant.

Efficiency of surface sterilization is additionally checked by transferring the surface sterilized samples in 5ml of distilled water and stirred suitably for 1 minute. An aliquot of 0.5 ml water obtained was inoculated on PDA (Potato Dextrose Agar) medium and incubated at $27^{\circ}\text{C} \pm 2$ for 7 days (Fig.1). Plates which exhibited negative result considered as successfully surface sterilized and used for isolation of endophytic fungi [30]. PDA plates were prepared for inoculation of fungal endophytes from stem, leaf and root, whereas the CDA plates were used to maintain the pure culture. The inoculated plates were set aside in the BOD Incubator ($25^{\circ}\text{C} \pm 2$) for 7-15 days and observed on a regular basis. Morphological and microscopic characters viz. growth pattern, colony color, surface texture, spore production, mycelial structure, etc. has been considered for the identification of the isolates up to genus level by referring to some standard monographs [10; 8; 5; 35].

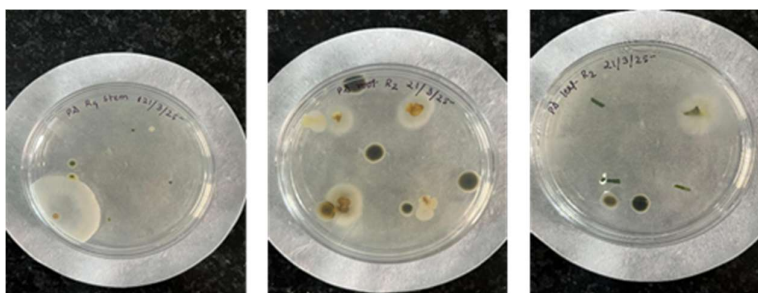


Figure1: Showing the growth of fungal endophytes on mother plate.

2.3 Antagonistic activity of the isolated fungal endophytes:

Dual culture assay for the isolated fungal endophytes against selected pathogens (Bacteria- *Escherichia coli* and fungi- *Fusarium* sp.) were done by following the modified protocol of Talapatra *et al.* (2017) [39]. The culture plates were incubated for 1-2 weeks at $36^{\circ}\text{C} \pm 2$ and $25^{\circ}\text{C} \pm 2$ respectively for bacterial and fungal pathogens against the isolated endophytic fungi. Over the course of work, plates were checked and measured the growth of both the pathogen and antagonist at regular intervals and the inhibition zones were recorded, if any.

2.4 Qualitative analysis of phytochemical screening:

To perform this experiment, the samples were set by cleaning with distilled water, drying under sun light for 2-3 weeks to get the fine powder through grinding.

2.4.1 Methanol extraction:

Plant material to execute this experiment was extracted using an 80% methanol solution at a 1:10 (w/v) ratio. The mixture was briefly shaken and macerated for 72 hours with intermittent agitation to enhance phytochemical release [15]. Following filtration, the extract was air-dried in Petri plates at room temperature. The dried residue was then scraped, transferred to Eppendorf tubes, and stored at 4°C until further analysis. This maceration technique effectively preserves bioactive constituents for subsequent evaluations.

2.4.2 Phytochemical screening:

The solution was used to precursory phytochemical screening following standard approaches constructed on various articles [14; 3; 41; 40].

2.4.2.1 Test for Phenolic: A few drops of FeCl_3 (10 %) solution was added in a 1 ml plant extract. The formation of green, red, purple, red, or blue-black colour indicated the presence of phenolic.

2.4.2.2 Test for Alkaloids (Wagner's Test): About 0.5ml of plant sample was treated with four to five drops of wagner's reagent and formation of reddish-brown precipitate (or coloration) was observed.

2.4.2.3 Test for Flavonoids (Alkaline reagent test): 2ml of extracts was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow colour, that becomes colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

2.4.2.4 Test for Tannins (Ferric Chloride Test): 1 ml of plant extract solution was stirred with 5 ml of distilled water. The formation of a blue, blue-black or blue-green colour or precipitation on the addition of (5%) FeCl_3 solution indicated the presence of tannins. The occurrence of blue-black colour showed the presence of gallic tannins and a blue-green colour indicated presence of catechol tannins.

2.4.2.5 Test for saponins (Foam Test): About 1 ml of plant extract solution was mixed with 3 ml of boiled water and shaken vigorously. If a foam was produced and it was stable for 1-2 minutes and persisted on warming, its evidence for the presence of saponin.

3. Results and Discussions:

3.1 Isolation and identification of Endophytic Fungi:

In the present study, three dominant fungal endophytes from the query plant viz. *Aspergillus* sp. (LP1), *Colletotrichum* sp. (SP1) and *Penicillium* sp. (SR1) were isolated (Table-1), these endophytic fungi also found to be common in various studies related to medicinal plants [13; 34]. Fungal endophytes, particularly species of *Aspergillus*, *Penicillium*, and *Colletotrichum*, play a crucial role in ecological systems and pharmaceutical

research due to their remarkable capacity to biosynthesize diverse secondary metabolites with significant antimicrobial, anticancer, and immunomodulatory activities [9; 23].

Table No.1: Morphology characteristics of macroscopic and microscopic observations of the isolates.

Plate No.	Macroscopic characteristics	Microscopic characteristics	Fungal isolates name
LP1	Colonies consists of compact white or yellow felt with a dense layer of brown to black conidiophores, the reverse is cream to yellow.	Conidiophores are protrusions from septate and hyaline hyphae, conidia heads appear radial and split into columns(biseriate). Mycelium also known as biofilm.	<i>Aspergillus</i> sp.
SP1	Characterized by oblong colonies vary in appearance ranging from fibrous, compact and cottony colonies, color usually white to greyish or greyish green.	Characterized by acervuli that produce dark brown setae and hyaline, aseptate conidia that germinate to produce appressoria.	<i>Colletotrichum</i> sp.
SR1	Colonies growing in shades of green, faster, mostly flat, filamentous and cottony in texture.	Multicellular mycelium mostly branched, multinucleated and septate long thread-like filamentous structure known as hyphae, conidia are produced from cells called phialide, sexual reproduction is formation of ascospores.	<i>Penicillium</i> sp.

3.2 Antagonistic activity against pathogens:

The isolated and identified endophytic fungi were subjected to dual culture assays against both bacterial (*Escherichia coli*) and fungal (*Fusarium* sp.) pathogens to evaluate their antagonistic potential (Fig. 2 & 3). In this study, we observed that *Colletotrichum* sp. excellent antibacterial ability as it completely overgrew the bacterial pathogen used. This aligns with studies showing *Colletotrichum* sp. producing bioactive metabolites effective against Gram-negative bacteria [12] whereas, *Aspergillus niger* and *Penicillium* sp. showed mutual inhibition, indicating competition for nutrients or antibiotic secretion [20]. *A. niger* and *Colletotrichum* sp. both showed inhibition of fungal pathogen *Fusarium* sp. upon contact most probable due to antibiosis or mycoparasitism [32], supporting Shang (2016) findings [34].

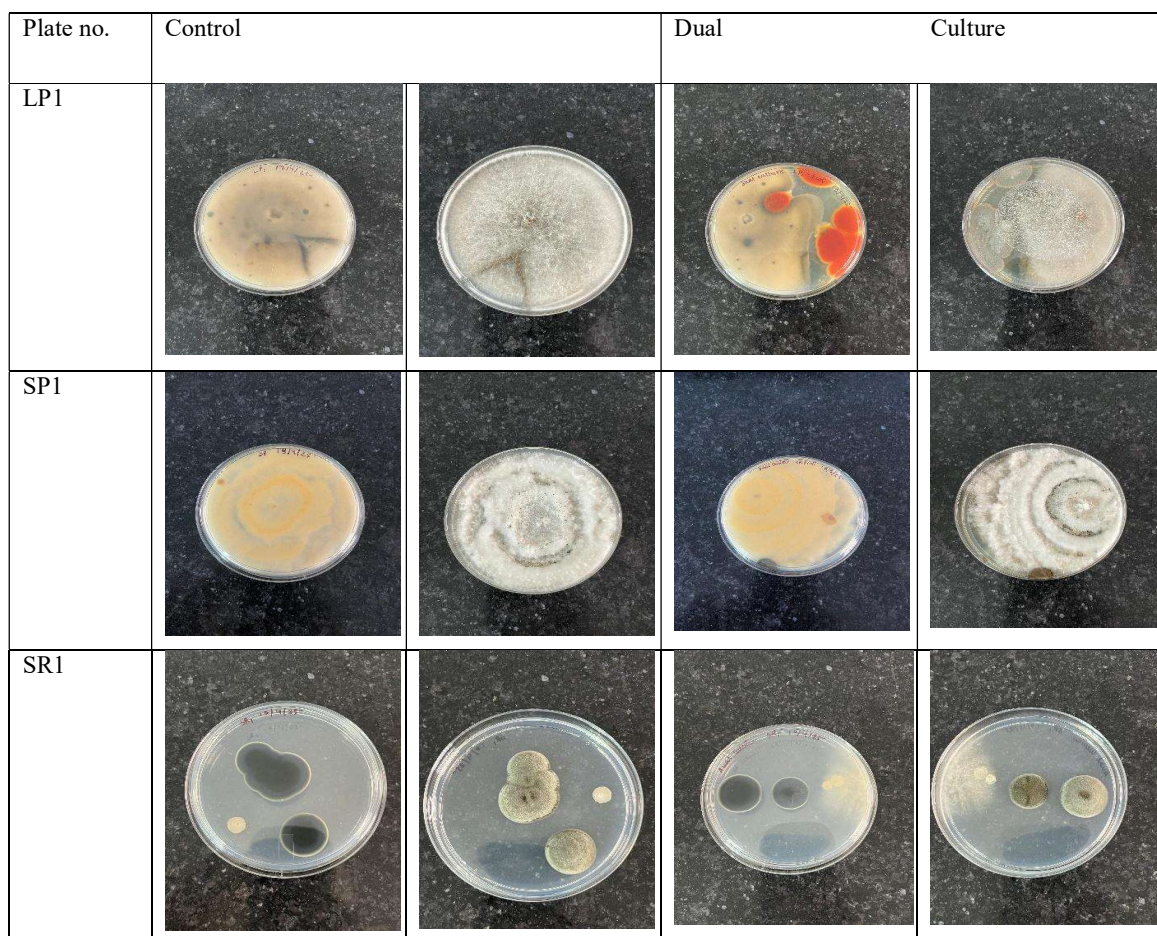


Figure 2: Dual culture assay against bacterial pathogen (*Escherichia coli*); LP1: *Aspergillus* sp., SP1: *Colletotrichum* sp. and SR1: *Penicillium* sp.





Figure 3: Dual culture assay against fungal pathogen (*Fusarium* sp.); LP1: *Aspergillus* sp., SP1: *Colletotrichum* sp. and SR1: *Penicillium* sp.

3.3 Phytochemical Screening:

The leaf and stem components of *Andrographis paniculata* were subjected to qualitative phytochemical screening, with 80% methanol employed as the extraction solvent (Fig. 4). The phytochemical screening using methanol extracts stems contained phenols, alkaloids and flavonoids, while leaves had alkaloids and flavonoids but no phenols (Table 2). These findings correlate with traditional uses of *A. paniculata* for antimicrobial and anti-inflammatory effects [2].

Solvent	Methanol				
Phytochemical	Phenol	Alkaloid	Flavonoid	Tannin	Saponin
Stem Extract					
Leaf Extract					

Figure 4: Qualitative phytochemical screening from the stem and leaf extract.

Table 2: Phytochemical assay for the various secondary metabolite with 80% methanol

Sl. No.	Phytochemical	Methanol	
		Stem	Leaf
1	Phenol	+	-
2	Alkaloid	+	+
3	Flavonoid	+	+
4	Tannin	-	-
5	Saponin	-	-

Present + ; Absent –

4. Conclusion:

The present study signifies the importance of *Andrographis paniculata* and the endophytic fungi associated with it. In this investigation we were able to isolate and identify the three dominant fungus species viz. *Aspergillus* sp., *Colletotrichum* sp., and *Penicillium* sp. which demonstrated outstanding antagonistic activity against *E. coli* and *Fusarium* sp. The presence of bioactive compounds such as phenols, flavonoids, and alkaloids were established through Phytochemical screening supporting the plant's traditional uses. The ability of endophytes to produce similar metabolites as the host underscores their potential in sustainable pharmaceutical and agricultural applications. This work advances our knowledge of the interactions between microbes and plants and encourages more research into endophytic fungus as potential sources of new bioactive substances.

Conflicts of Interest

There is no known conflict of interest between the authors that would influence this work in future.

References:

- [1]. Adhikari M, and Mukhopadhyay M (2021). Potentials of endophytes of *Andrographis paniculata* for the production of plant growth promoters, enzymes and antimicrobial compounds. *SAARC Journal of Agriculture*, 19(2), 157-170.
- [2]. Akbar S (2011). *Andrographis paniculata*: a review of pharmacological activities and clinical effects. *Alternative Medicine Review*, 16(1), 66-77.
- [3]. Alamzeb M, Khan MR, Ali S, Shah SQ, and Mamoon UR (2013). Antimicrobial properties of extracts and compounds isolated from *Berberis jaeschkeana*. *Bangladesh Journal of Pharmacology*, 8(2), 107–109. <https://doi.org/10.3329/bjp.v8i2.13551>.
- [4]. Barnett HL, Hunter BB. 1972 – Illustrated Genera of Imperfect Fungi. 3rd ed. Burgess Publishing Company. Printed in United States of America 241.
- [5]. Bayman P, Lebron LL, Tremblay RL, and Lodge DJ (1997). Variation in endophytic fungi from roots and leaves of *Lepanthes* (Orchidaceae). *The New Phytologist*, 135(1), 143-149.
- [6]. de Siqueira VM, Conti R, de Araújo JM, and Souza-Motta CM (2011). Endophytic fungi from the medicinal plant *Lippia sidoides* Cham. and their antimicrobial activity. *Symbiosis*, 53, 89-95.
- [7]. Diniz LRL, Perez-Castillo Y, Elshabrawy HA, Filho CdSMB, and de Sousa DP (2021). Bioactive Terpenes and Their Derivatives as Potential SARS-CoV-2 Proteases Inhibitors from Molecular Modeling Studies. *Biomolecules*, 11(1), 74. <https://doi.org/10.3390/biom1101007>.
- [8]. Domsch KH, Gams W, Anderson TH. 1980 – Compendium of soil fungi. Academic Press, London.
- [9]. Elfita E, Munawar M, Muharni M, and Ivantri I (2015). Chemical Constituents from an Endophytic Fungus *Aspergillus* sp (SbD5) Isolated from Sambilloto (*Andrographis paniculata* Nees). *Microbiology Indonesia*, 9(2), 6-6.
- [10]. Ellis MB. 1976 – More Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, UK
- [11]. Firdous J, Latif NA, Mona R, Mansor R, and Muhamad N (2020). *Andrographis paniculata* and its endophytes: a review on their pharmacological activities. *Research Journal of Pharmacy and Technology*, 13(4), 2029-2032.
- [12]. Febria F, Suryelita S, and Riga R (2022). Antibacterial Activity and phytochemical screening of the fraction of endophytic fungus derived from sambilloto flowers (*Andrographis paniculata* (Burm. f.) Nees). *Jurnal Sains Natural*, 12(3), 134-142.
- [13]. Gajalakshmi S, Iswarya V, Ashwini R, Bhuvaneshwari M, Mythili S, and Sathivelu A (2012). Secondary metabolite production by endophytic fungi isolated from *Andrographis paniculata*. *Int J Biomed Sci Technol*, 5, 12-17.
- [14]. Harborne AJ (1998). *Phytochemical methods a guide to modern techniques of plant analysis*. Springer science & business media.
- [15]. Handa S, Khanuja SPS, Longo G, and Rakesh DD (2008). *Extraction technologies for medicinal and aromatic plants* (1st ed., No. 66). United Nations Industrial Development Organization (UNIDO) and the International Centre for Science and High Technology.
- [16]. Jia M, Chen L, Xin HL, Zheng J, Rahman K, Han T, and Qin LP (2016). A friendly relationship between endophytic fungi and medicinal plants: a systematic review. *Frontiers in microbiology*, 7, 906.

- [17]. Khaing TT, Aye M, and Oo AK (2023). Isolation of endophytic fungi from *andrographis paniculata* all. Ex nees and their antimicrobial activities. *Journal of the Myanmar Academy of Arts and Science*, XXI(3), 333-343.
- [18]. Li N, Xu D, Huang RH, Zheng Y, Liu Y, Hu BS, and Du Q (2022). A new source of diterpene lactones from *Andrographis paniculata* (Burm. f.) Nees—Two endophytic fungi of *Colletotrichum* sp. with antibacterial and antioxidant activities. *Frontiers in Microbiology*, 13, 819770. doi:10.3389/fmicb.2022.819770. eCollection 2022.
- [19]. Li G, Kusari S, Lamshöft M, Schöffler A, Laatsch H, and Spiteller M (2014). Antibacterial secondary metabolites from an endophytic fungus, *Eupenicillium* sp. LG41. *Journal of natural products*, 77(11), 2335-2341.
- [20]. Latz MA, Jensen B, Collinge DB, and Jørgensen HJ (2018). Endophytic fungi as biocontrol agents: elucidating mechanisms in disease suppression. *Plant Ecology & Diversity*, 11(5-6), 555-567
- [21]. Maheswari NU, and Kaviyarasi P (2021). Antagonistic activity and phytochemical screening of endophytic fungi isolated from medicinal plants. *International Journal of Botany Studies*, 6(5), 1271-1276.
- [22]. Masumi S, Mirzaei S, Zafari D, and Kalvandi R (2015). Isolation, identification and biodiversity of endophytic fungi o *Thymus*. *Progress in Biological Sciences*, 5(1), 43-50.
- [23]. Mamangkey J, Mendes LW, Mustopa AZ, Hartanto A (2024) Endophytic *Aspergillii* and *Penicillii* from medicinal plants: a focus on antimicrobial and multidrug resistant pathogens inhibitory activity. *BioTechnologia* (Pozn), 29;105(1):83-95. doi: 10.5114/bta.2024.135644. PMID: 38633888; PMCID: PMC11020150.
- [24]. Mohana Kumara P, Zuehlke S, Priti V, Ramesha T, Shweta S, Ravikanth G, and Uma Shaanker R (2012). *Fusarium proliferatum*, an endophytic fungus from *Dysoxylum binectariferum* Hook. f, produces rohitukine, a chromane alkaloid possessing anti-cancer activity. *Antonie Van Leeuwenhoek*, 101, 323-329.
- [25]. Nessa F, Shamsi S, and Al Noman MA (2023). Morphological and molecular characterization of endophytic fungi isolated from *Andrographis paniculata* (Burm. f.) Wall. ex Nees and *Centella asiatica* (L.) Urban. *Bangladesh Journal of Plant Taxonomy*, 30(1), 111-122.
- [26]. Okhwarobo A, Falodun JE, Erharuyi O, Imieje V, Falodun A, and Langer P (2014). Harnessing the medicinal properties of *Andrographis paniculata* for diseases and beyond: a review of its phytochemistry and pharmacology. *Asian Pacific journal of tropical disease*, 4(3), 213-222. [https://doi.org/10.1016/S2222-1808\(14\)60509-0](https://doi.org/10.1016/S2222-1808(14)60509-0)
- [27]. Perveen S, and Al-Taweel A (2018). Introductory chapter: terpenes and terpenoids. *Terpenes and terpenoids*, 1, 1-12.
- [28]. Puri SK, Habbu PV, Kulkarni PV, and Kulkarni VH (2019). Investigations on Screening of Endophytic Fungal Fractions of *Andrographis Paniculata* (Burm. F.) Wall Nees. Leaves in Paracetamol and Ethanol Induced Hepatotoxicity. *RGUHS Journal of Pharmaceutical Sciences*, 9(4).
- [29]. Sabovljevic A, Sabovljevic M, and Jockovic N (2009). In vitro culture and secondary metabolite isolation in bryophytes. *Protocols for in vitro cultures and secondary metabolite analysis of aromatic and medicinal plants*, 117-128.
- [30]. Schulz B, Wanke U, Draeger S, and Aust HJ (1993). Endophytes from herbaceous plants and shrubs: effectiveness of surface sterilization methods. *Mycological research*, 97(12), 1447-1450.
- [31]. Schulz B, Boyle C, Draeger S, Römmert AK, and Krohn K (2002). Endophytic fungi: a source of novel biologically active secondary metabolites. *Mycological research*, 106(9), 996-1004.
- [32]. Segaran G, and Sathiavelu M (2023). Fungicidal and plant growth-promoting traits of *Lasiodiplodia pseudotheobromae*, an endophyte from *Andrographis paniculata*. *Frontiers in Plant Science*, 14, 1125630.
- [33]. Selim KA, El-Beih AA, AbdEl-Rahman T, and El-Diwany AI (2012). Biology of endophytic fungi. *Current Research in Environmental & Applied Mycology*, 2(1), 31–82.
- [34]. Shang TW (2017). *Diversity and Bioactivities of Endophytic Fungi from Medicinal Plant Andrographis paniculata (Hempedu Bumi)* (Doctoral dissertation, Monash University).
- [35]. Subramanian CV. 1971 – Hyphomycetes: an account of Indian species, except Cercosporae. Indian Council of Agricultural Research, New Dehli.
- [36]. Sun, X, and Guo LD (2012). Endophytic fungal diversity: review of traditional and molecular techniques. *Mycology*, 3(1), 65-76.
- [37]. Suryelita S, Riga R, Etika SB, Ulfah M, and Artasasta MA (2021). Antibacterial screening of endophytic fungus xylaria sp. Derived from *Andrographis paniculata* (sambiloto). *Open Access Macedonian Journal of Medical Sciences*, 9(A), 971-975.

- [38]. Syamsia S, Idhan A, Latifah H, Noerfityani N, and Akbar A (2021). Alternative medium for the growth of endophytic fungi. In IOP Conference Series: Earth and Environmental Science (Vol. 886, No. 1, p. 012045). IOP Publishing.
- [39]. Talapatra K, Das AR, Saha AK, and Das P (2017). In vitro antagonistic activity of a root endophytic fungus towards plant pathogenic fungi. *Journal of Applied Biology and Biotechnology*, 5(2), 68-71.
- [40]. Talukdar A, and Chaudhary B (2010). Phytochemical screening of ethanolic extracts of *Rubia cordifolia*. *Pharma Biology Science*, 1(4), 530–536.
- [41]. Thusa R, and Mulmi S (2017). Analysis of phytoconstituents and biological activities of different parts of *Mahonia nepalensis* and *Berberis aristata*. *Nepal Journal of Biotechnology*, 5(1), 5–13. <https://doi.org/10.3126/njb.v5i1.18864>
- [42]. Xu D, Li N, Gu YQ, Huang J, Hu BS, Zheng JY, and Du Q (2023). Endophytic fungus *Colletotrichum* sp. AP12 promotes growth physiology and andrographolide biosynthesis in *Andrographis paniculata* (Burm. f.) Nees. *Frontiers in Plant Science*, 14, 1166803.